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 NEWS  9  DEC 17   ELCOM reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  10 DEC 17   COMPUAB reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
 NEWS  11 DEC 17   SOLIDSTATE reloaded; updating to resume; current-awareness
                   alerts (SDIs) affected
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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005

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=> s antifreeze protein and activity
   7 FILES SEARCHED...
L1          870 ANTIFREEZE PROTEIN AND ACTIVITY

=> s recrystallization inhibition?
L2           80 RECRYSTALLIZATION INHIBITION?

=> s thermal hysteresis protein adj(impart antifreeze property)
MISSING OPERATOR 'ADJ(IMPART'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s thermal hysteresis protein with (impart antifreeze property?)
MISSING OPERATOR 'WITH (IMPART'
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=> s l1 and l2
L3           32 L1 AND L2

=> s l3 and (method)
L4           17 L3 AND (METHOD)

=> d l4 ti abs ibib tot

L4   ANSWER 1 OF 17  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
TI   A facile method for determining ice recrystallization
     inhibition by antifreeze proteins.
AB   Ice recrystallization, the growth of large ice crystals at the expense of
     small ones, stresses freeze tolerant organisms and causes spoilage of
     frozen foods.  This process is inhibited by antifreeze proteins (AFPs).
     Here, we present a simple method for determining the ice
     recrystallization inhibition (RI) activity of
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an AFP under physiological conditions using 10 mul glass capillaries. Serial dilutions were prepared to determine the concentration below which RI **activity** was no longer detected, termed the RI endpoint. For type III AFP this was 200 nM. The capillary **method** allows samples to be aligned and viewed simultaneously, which facilitates RI endpoint determination. Once prepared, the samples can be used reproducibly in subsequent RI assays and can be archived in a freezer for future reference. This **method** was used to detect the elution of type III AFP from a Sephadex G-75 size-exclusion column. RI **activity** was found at the expected Ve for a 7 kDa protein and also unexpectedly in the void volume.

ACCESSION NUMBER: 2004:64469 BIOSIS
DOCUMENT NUMBER: PREV200400065777
TITLE: A facile **method** for determining ice **recrystallization inhibition** by antifreeze proteins.
AUTHOR(S): Tomczak, Melanie M.; Marshall, Christopher B.; Gilbert, Jack A.; Davies, Peter L. [Reprint Author]
CORPORATE SOURCE: Department of Biochemistry and Protein Engineering Network of Centres of Excellence, Queens University, Kingston, ON, K7L 3N6, Canada
daviesp@post.queensu.ca
SOURCE: Biochemical and Biophysical Research Communications, (November 28 2003) Vol. 311, No. 4, pp. 1041-1046. print.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Jan 2004
Last Updated on STN: 28 Jan 2004

L4 ANSWER 2 OF 17 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
TI Stable, high-level expression of a type I **antifreeze protein** in Escherichia coli.
AB The type I antifreeze proteins are simple amphipathic helical proteins found in abundance in polar fish species, where they act to prevent freezing of internal fluids by a mechanism of noncolligative freezing point depression. Large-scale production of these proteins for research and biotechnological purposes has been hampered by their apparent instability when expressed in heterologous host systems. This has necessitated their production as fusion proteins, in polymeric form, or as proproteins for secretion, with the concomitant necessity for postpurification processing to generate the mature form of the protein. We have successfully expressed a recombinant variant of type I **antifreeze protein** (rAFP) in Escherichia coli using the inducible T7 polymerase transcription expression system. The rAFP contains five copies of the 11 amino acid ice-binding repeat motif found in all type I antifreeze proteins. The protein accumulates to high levels intracellularly in the form of inclusion bodies, with no apparent degradation by the cellular proteolytic machinery. We have devised a simple and rapid purification protocol for this recombinant type I **antifreeze protein** which does not require cellular fractionation, purification of the inclusion bodies, or chromatographic steps. This protocol may be of general use for this class of protein. The protein displays all three activities common to these proteins: **recrystallization inhibition**, noncolligative freezing point depression, and modification of the morphology of single ice crystals in solution.

ACCESSION NUMBER: 1999:305223 BIOSIS
DOCUMENT NUMBER: PREV199900305223
TITLE: Stable, high-level expression of a type I **antifreeze protein** in Escherichia coli.
AUTHOR(S): Solomon, Robert G. [Reprint author]; Appels, Rudi [Reprint author]

CORPORATE SOURCE: CSIRO Plant Industry and Quality Wheat CRC Ltd, Canberra, ACT, 2601, Australia
SOURCE: Protein Expression and Purification, (June, 1999) Vol. 16, No. 1, pp. 53-62. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English.
ENTRY DATE: Entered STN: 12 Aug 1999
Last Updated on STN: 12 Aug 1999

L4 ANSWER 3 OF 17 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
TI Tracking the profile of a specific **antifreeze protein** and its contribution to the thermal hysteresis **activity** in cold hardy insects.
AB This study summarizes some important new directions in research on **antifreeze protein** biosynthesis and regulation. It describes the recent development and availability of essential biochemical and cellular tools that make possible more direct cellular investigations, and an assessment of the relationship between thermal hysteresis protein (THP) levels and antifreeze **activity** (both thermal hysteresis and **recrystallization inhibition** (RI)). These tools include: 1) the isolation of a specific THP of high **activity** (designated Tm 12.86), and an additional endogenous activating factor of this **antifreeze protein**; 2) the ability to track the cellular and secretory patterns of Tm 12.86 immunologically; 3) the use of an in vitro fat body cell culture system for direct investigation of cellular events. and, 4) a means of quantifying RI behavior of purified Tm 12.86, and samples of unknown concentrations of THPs, to provide a more sensitive detection **method** for antifreeze **activity** at scaled down values associated with the in vitro system. In combination, these studies indicate that the adaptation mechanisms contributing to the overall **antifreeze protein** response in a cold hardy insect involves a complex interaction between antifreeze proteins and endogenous activators of these proteins. With the availability of these key tools, the details of a precise and seasonal regulation of these **antifreeze protein**/activator interactions, which ultimately generate an efficient cold hardy response, now have the potential to be worked out.
ACCESSION NUMBER: 1996:538806 BIOSIS
DOCUMENT NUMBER: PREV199699261162
TITLE: Tracking the profile of a specific **antifreeze protein** and its contribution to the thermal hysteresis **activity** in cold hardy insects.
AUTHOR(S): Horwath, Kathleen L. [Reprint author]; Easton, Christopher M.; Poggioli., George J., Jr.; Myers, Kevin; Schnorr, Ingrid L.
CORPORATE SOURCE: Dep. Biol. Sci., Binghamton Univ., Binghamton, NY 13902-6000, USA
SOURCE: European Journal of Entomology, (1996) Vol. 93, No. 3, pp. 419-433.
ISSN: 1210-5759.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Dec 1996
Last Updated on STN: 10 Dec 1996

L4 ANSWER 4 OF 17 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI New cDNA polynucleotide encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing antifreeze protection to improve the quality of food.
AN 2002-090137 [12] WPIDS
AB WO 200194378 A UPAB: 20020221
NOVELTY - A cDNA polynucleotide (I) comprising a nucleotide sequence for

encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a mRNA polynucleotide (II) comprising a nucleotide sequence for encoding thermal hysteresis proteins derived from the Tenebrionoidea Superfamily transcribed from (I);

(2) a DNA or RNA probe having a sequence complementary or identical to a sequence of contiguous nucleotides for at least a portion of (I);

(3) a recombinant vector containing (I);

(4) a thermal hysteresis protein, preferably an endogenous Type III anti-freeze proteins, derived from the Tenebrionoidea Superfamily which lowers the freezing point of a solution without effecting the melting point of the solution;

(5) a consensus sequence with a nucleotide sequence selected from one of the four 481 nucleotide sequences (S1-S4) defined in the specification;

(6) a consensus sequence with an amino acid sequence selected from the 133 (S5), 134 (S6), another 134 (S7), another 134 (S8) amino acid sequence defined in the specification;

(7) a consensus sequence with the 133 amino acid sequence (S9) defined in the specification;

(8) a primer having a nucleotide sequence selected from P1-P3;

(9) a **method** (M1) for producing a polypeptide having antifreeze properties comprising forming a cloning vector with a Tm 12.86 family member gene encoding an antifreeze polypeptide, transferring genes of the cloning vector into DNA of host cell to create a transformed cell, expressing a mRNA sequence and a translated amino acid sequence from the recombinant expression vector, the sequence being isoforms of the Tm 12.86 T. molitor antifreeze polypeptide;

(10) a **method** (M2) for providing antifreeze or **recrystallization inhibition** properties to a subject formulation comprising incorporating at least 0.1 micrograms to 1 mg of an activated polypeptide into 1 ml of a subject formulation to obtain **recrystallization inhibition** or 1 mg to 25 mg of the activated polypeptide into 1 ml of a subject formulation to thermal hysteresis;

(11) a Tm 12.86 antibody/antiserum;

(12) a **recrystallization inhibition method** (M3) for determining the presence, relative concentration, and/or **activity** of thermal hysteresis proteins comprising providing a proteinaceous composition in a solvent to form a test solution, flash freezing the solution, raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within the solution, maintaining the frozen solution at the annealing temperature for a length of time sufficient to allow for recrystallization, monitoring the ice crystal grain size changes over time, and determining the presence of functional thermal hysteresis proteins in the solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution;

(13) a **method** for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process defined in M3; and

(14) a **method** for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process defined in M3.

CGCGGATCCCTCACCGACGAACAG (P1);

GAGAGGATAACTAATTGAGCTCGCC (P2); and

CGCGGATCCCTGACCGAGGCACAA (P3).

USE - The activated anti-freeze protein is incorporated into:

(a) plant, produce or fish in an amount sufficient to provide antifreeze protection;

(b) a region of a target tissue in an amount sufficient to provide **antifreeze protein** controlled limited tumor cell or target tissue cryoinjury during cryosurgery;

(c) hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic or hypothermic preservation of cells and tissues by incorporating the protein into the cells, tissue, or cell membranes in a controlled amount sufficient to provide antifreeze protection;

(d) de-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces such as a road, aircraft, household products, cosmetic products, machinery and plant surfaces; or

(e) a food product in an amount sufficient to provide antifreeze protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage.

The polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization. The Tm 12.86 antibody/antiserum is used as a screening device to identify positive recombinant plaques containing cloned inserts capable in an expression vector system to produce recombinant products recognized by the antibody/antiserum. The Tm 12.86 antibody/antiserum which is also used as a screening device to screen cDNA libraries in an expression system, including cross-species cDNA libraries to identify homologous sequences in other species.

M3 is used for concurrent multiple sample testing of solutions which includes the 'sandwich' **method**; and application via a 96 well plate device (all claimed).

Dwg.0/8

ACCESSION NUMBER: 2002-090137 [12] WPIDS
DOC. NO. CPI: C2002-027870
TITLE: New cDNA polynucleotide encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing antifreeze protection to improve the quality of food.
DERWENT CLASS: C06 D16
INVENTOR(S): HORWATH, K L; MEYERS, K L; EASTON, C M; MYERS, K L
PATENT ASSIGNEE(S): (EAST-I) EASTON C M; (HORW-I) HORWATH K L; (MYER-I) MYERS K L; (UYNY) UNIV NEW YORK STATE RES FOUND; (MEYE-I) MEYERS K L
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001094378	A1	20011213	(200212)*	EN	231
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001075389	A	20011217	(200225)		
US 2002172951	A1	20021121	(200279)		
US 2002173024	A1	20021121	(200279)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001094378	A1	WO 2001-US18532	20010607
AU 2001075389	A	AU 2001-75389	20010607
US 2002172951	A1 Provisional	US 2000-210446P	20000608
		US 2001-876348	20010607
US 2002173024	A1 Provisional	US 2000-210446P	20000608

US 2001-876796 20010607

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001075389	A Based on	WO 2001094378

PRIORITY APPLN. INFO: US 2000-210446P 20000608; US 2001-876348 20010607; US 2001-876796 20010607

L4 ANSWER 5 OF 17 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

TI New cDNA polynucleotide encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing antifreeze protection to improve the quality of food;
phagemid vector-mediated recombinat protein gene transfer and expression in bacterium cell, transgenic plant, transgenic fish and transgenic animal for cold climatization enhancement

AN 2002-07231 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A cDNA polynucleotide (I) comprising a nucleotide sequence for encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a mRNA polynucleotide (II) comprising a nucleotide sequence for encoding thermal hysteresis proteins derived from the Tenebrionoidea Superfamily transcribed from (I); (2) a DNA or RNA probe having a sequence complementary or identical to a sequence of contiguous nucleotides for at least a portion of (I);(3) a recombinant vector containing (I); (4) a thermal hysteresis protein, preferably an endogenous Type III anti-freeze proteins, derived from the Tenebrionoidea Superfamily which lowers the freezing point of a solution without effecting the melting point of the solution; (5) a consensus sequence with a nucleotide sequence selected from one of the four 481 nucleotide sequences (S1-S4) defined in the specification; (6) a consensus sequence with an amino acid sequence selected from the 133 (S5), 134 (S6), another 134 (S7), another 134 (S8) amino acid sequence defined in the specification; (7) a consensus sequence with the 133 amino acid sequence (S9) defined in the specification; (8) a primer having a nucleotide sequence selected from P1-P3; (9) a **method** (M1) for producing a polypeptide having antifreeze properties comprising forming a cloning vector with a Tm 12.86 family member gene encoding an antifreeze polypeptide, transferring genes of the cloning vector into DNA of host cell to create a transformed cell, expressing a mRNA sequence and a translated amino acid sequence from the recombinant expression vector, the sequence being isoforms of the Tm 12.86 T. molitor antifreeze polypeptide; (10) a **method** (M2) for providing antifreeze or **recrystallization inhibition** properties to a subject formulation comprising incorporating at least 0.1 micrograms to 1 mg of an activated polypeptide into 1 ml of a subject formulation to obtain **recrystallization inhibition** or 1 mg to 25 mg of the activated polypeptide into 1 ml of a subject formulation to thermal hysteresis; (11) a Tm 12.86 antibody/antiserum; (12) a **recrystallization inhibition method** (M3) for determining the presence, relative concentration, and/or **activity** of thermal hysteresis proteins comprising providing a proteinaceous composition in a solvent to form a test solution, flash freezing the solution, raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within the solution, maintaining the frozen solution at the annealing temperature for a length of time sufficient to allow for recrystallization, monitoring the ice

crystal grain size changes over time, and determining the presence of functional thermal hysteresis proteins in the solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution; (13) a **method** for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process defined in M3; and (14) a **method** for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process defined in M3. CGCGGATCCCTCACCGACGAACAG (P1); GAGAGGATAACTAATTGAGCTCGCC (P2); and CGCGGATCCCTGACCGAGGCACAA (P3).

BIOTECHNOLOGY - Preferred Protein: The thermal hysteresis protein is from the group consisting of Tm 12.86, Tm 2.2, Tm 3.4, Tm 3.9, Tm 7.5, Tm 2.3, Tm 13.17, Tm 12.84 or their isoforms. The thermal hysteresis protein has an amino acid sequence selected from one of the 39 sequenced defined in the specification or S5-S9. Preferred Nucleic Acid: In (I) and (II), the nucleotide sequence is selected from one of the 18 nucleotide sequences (S10) defined in the specification, or S1-S4, or their respective complements. The nucleotide sequence further includes a 5' end selected from non-his/signal plus, non-his/signal minus, his/signal plus and his/signal minus. Preferred **Method**: M1 further comprises isolating the amino acid sequence and establishing **antifreeze protein activity** for the amino acid sequence. The amino acid sequence is selected from S5-S9. The polypeptide has an apparent molecular weight from about 11000 to 25000 Daltons. Isolating the amino acid sequence comprises extraction from inclusion bodies within the transformed host bacterial cell. Establishing **activity** further comprises denaturing and extracting proteins from the transformed cells followed by renaturizing and purifying the polypeptide, followed by further denaturing and refolding. The **activity** step provides antifreeze polypeptide **activity** as measured by thermal hysteresis or antifreeze specific **recrystallization inhibition**. In M2, the activated polypeptide provides a non-colligative freezing point depression and an antifreeze specific inhibition of recrystallization. M2 further comprising an enhancing activator species. The activator is an endogenous activator from T. molitor or Tm 12.86 antisera. In M3, the solvent selected from water, saline, phosphate buffered saline (PBS), or other isoosmotic inorganic or organic solutions. Two or more control solutions are used, where one control is the solvent and the other is a control for non-specific **recrystallization inhibition** effects. The proteinaceous composition is selected from antifreeze polypeptides (such as a thermal hysteresis protein, e.g. purified Tm 12.86 or Tm 12.84, with a known **activity**), antifreeze glycopeptides, recombinant antifreeze polypeptides, recombinant antifreeze glycopeptides, synthetic antifreeze polypeptides analogs, synthetic antifreeze glycopeptide analogs, cell culture products, activator, recombinant bacterial products, recombinant products, uncharacterized plant products and transgenic plant products. Alternatively, the proteinaceous composition has unknown functional **antifreeze protein activity**. The protein composition of Tm 12.86 is 0.5 micrograms to 25 micrograms/ml. The protein content is less than or equal to 1 mg/ml in saline and PBS, and less than or equal to 0.005 mg/ml in water. The **recrystallization inhibition method** is carried out under conditions to eliminate non-thermal hysteresis protein induced **recrystallization inhibition** effects. The conditions in saline are at -6 degrees Centigrade for 30 minutes with total protein content less than or equal to 1 mg/ml; or in water at -2 degrees Centigrade for 2 hours with total protein content less than or equal to 0.005 mg/ml. The **recrystallization inhibition method** is carried out under conditions to avoid hyperosmotic solutions. Monitoring of ice crystal grain size changes over time is by photomicroscopy, digital or video imaging. The quantitative data is

collected by measurement of the mean largest ice grain size for both the test and control solutions to provide a basis for numerical assessment of the extent of **recrystallization inhibition** occurring.
The composite mlgs are obtained for the test solution and the control solution, which are then statistically compared. The quantitative data collection is collected by assessment using a densitometer of light transmitted through a low magnification full view photographic negative of frozen sample wafer; absorbance peaks for the test solution is evaluated for maximum amplitude and statistically compared with the control solution. The dilution profile of the test solution is obtained over a wide dilution range until mlgs, or another quantifiably assessed response variable, are no longer significantly different from the saline/PBS and/or non-THP containing proteinaceous control solutions. The composite mlgs, or absorbance peak area (light scattering), or computer generated units (digital/video imaging)) are calculated for the test solution and plotted as a function of the logarithm of sample concentration, with replicate dilution series tested, and compared to control solution baseline. The linear regression analyses is used to approximate the linear portion of the dilution profile, with application of a transforming function (arcsine((mlgs)0.5) verses log(dilution)) to mlgs to limit inherent curvature of dilution plots caused by the 'leveling off' of mlgs values for both very dilute and very concentrated thermal hysteresis protein samples. The linear regression analyses provides the basis for development of a numerical factor (RI factor) describing the **activity** of the test solution with respect to **recrystallization inhibition** capability. The RI factor is equal to the absolute value of the logarithm of the minimum test solution dilution required to eliminate **recrystallization inhibition activity**. The RI factor is a measure of test solution **recrystallization inhibition** strength, according to the assessed exponential factor required for sufficient dilution of test solution to lose **recrystallization inhibition activity**, and providing a relative assessment of functional thermal hysteresis concentration within the test solution. The RI factor provides a relative assessment of functional thermal hysteresis protein concentration, and comparisons of various test solutions concentrations given translational shifts along the X axis. The regression line slope and Y-intercept reflect the **recrystallization inhibition** potency of a given test solution, thermal hysteresis protein species, recombinant thermal hysteresis protein product, synthetic thermal hysteresis analogue, or the like. The slope comparisons and shifts along Y-intercept provide relative potency comparisons between test solutions, thermal hysteresis species and the like. The expected concentrations of Tm 12.86 producing equivalent RI profiles are deduced, and provide reference interpretations of the test solution(s) functional **activity**(ies) to an **antifreeze protein** of known characterized parameters experimentally measured. The **activity** and potency of the test solution may include a combination of more than one type of thermal hysteresis protein, and/or thermal hysteresis protein plus activator solutions such as in test solution of hemolymph, or artificial solutions containing known amounts of purified thermal hysteresis protein with an activator supplement. M3 further comprises mathematical modeling of the **recrystallization inhibition** process with prediction of effects on slope and Y-intercept and log/log transformations for test solution mlgs data and analysis. The relationship between RI factors and thermal hysteresis levels for functionally active test solutions are described by equation: RI factor = 1.428 LOG(TH) + 3.703. A random sampling **method** is used for data collection generating mlgs which significantly eliminates the impact of intrasample ice crystal grain heterogeneity at high annealing temperature and with saline/PBS solvents.

USE - The activated anti-freeze protein is incorporated into: (a) plant, produce or fish in an amount sufficient to provide antifreeze

protection; (b) a region of a target tissue in an amount sufficient to provide **antifreeze protein** controlled limited tumor cell or target tissue cryoinjury during cryosurgery; (c) hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic or hypothermic preservation of cells and tissues by incorporating the protein into the cells, tissue, or cell membranes in a controlled amount sufficient to provide antifreeze protection; (d) de-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces such as a road, aircraft, household products, cosmetic products, machinery and plant surfaces; or (e) a food product in an amount sufficient to provide antifreeze protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage. The polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization. The Tm 12.86 antibody/antiserum is used as a screening device to identify positive recombinant plaques containing cloned inserts capable in an expression vector system to produce recombinant products recognized by the antibody/antiserum. The Tm 12.86 antibody/antiserum which is also used as a screening device to screen cDNA libraries in an expression system, including cross-species cDNA libraries to identify homologous sequences in other species. M3 is used for concurrent multiple sample testing of solutions which includes the 'sandwich' **method**; and application via a 96 well plate device (all claimed).

EXAMPLE - mRNA isolated from winter-acclimated whole animal and fat body of T. molitor were used as starting material to construct cDNA libraries. The ZAP express cDNA synthesis kit purchased from Stratagene was used for synthesis of cDNA. The detailed protocols suggested by the manufacturer were followed. The above cDNAs were applied to the Sephacryl 5-500 spin column to get rid of small pieces and uncomplete cDNA. Fractions were collected after each spin. Then each fraction was precipitated and ligated to the ZAP express vector arms, which generated libraries with different size of cDNA inserts. The ligated ZAP express vector was packaged into lambda phage particles using ZAP express cDNA Glgapack Gold Cloning Kit (Stratagene), i.e. packaging the vector with lambda coat protein to have viable phage **activity**. The cDNA libraries were amplified by plating on NZY plates with XL 1-blue MRF' strain (Stratagene). Phages were plated at high density with 50000 plaque forming units (pfu) per plate (150 mm) as recommended by Stratagene in the PicoBlue immunoscreening kit. Briefly, the XL1-blue MRF' cells were cultured overnight in NZY medium (5 g NaCl, 2 g MgSO4.7H2O, 5 g yeast extract, 10 g NZ amine (casein hydrolysate), 15 g agar per liter at pH 7.5) supplemented with 10 mM MgSO4 and 0.2%(v/v) of maltose. When the cell density reached OD600 of 1.0 the cells were pelleted and resuspended with sterilized 10 mM MgSO4 and diluted to a final OD600 of 0.5. A portion of this XL1-Blue MRF' cell suspension was mixed with phages and incubated for 15 minutes at 37 degrees Centigrade, then the 30 melted NZY top agar (5 g NaCl, 2 g MgSO4.7H2O, 5 g yeast extract, 10 g NZ amine and 0.7 %(v/v) agarose, pH 7.5) was added and mixed. The mixture was immediately poured onto the surface of a pre-prepared agar plates and left to solidify at room temperature. The agar plates were then incubated at 42 degrees Centigrade for 5 hours. During incubation the nitrocellulose membranes (Stratagene) were submerged in 10 mM IPTG (isopropyl-1-thio-Beta-D-galactopyranoside) solution. After completely wetting the nitrocellulose membranes, they were placed on Whatman 3 mm paper to air dry. When small plaques became visible in plates, the plates were covered with the treated nitrocellulose membranes and incubated for another 3-5 hours or overnight at 37 degrees Centigrade. The expression of cDNA in the vector is induced by IPTG absorbed in the membrane and the expressed proteins would be transferred to the membrane via plaque lift process. The lifted nitrocellulose membranes were washed in phosphate buffered saline (PBS) buffer and subjected to immunoblot screening. The nitrocellulose membranes obtained during the phage lift were washed in

PBS (0.002 M KCl, 0.14 M NaCl, 0.01 M Na2HPO4, 0.0015M KH2PO4, pH 7.2) after lifting. The wash was usually carried out for 3 times with shaking, each lime for 5 mm. The membrane was first blocked with fresh 5 % nonfat dry milk in PBS buffer for one hour with gentle agitation and then washed with PBS as described above. To block the possible endogenous perixodases in the membrane, the membrane then was incubated with fresh 0.5 % H2O2 for 5-30 mm and followed by washing with PBS for three times. Next, the membrane was incubated in the primary antibody against Tm 12.86 kD **antifreeze protein** (primary antibody serum was diluted at 1:1000 with PBS) for one to two hours with gentle shaking at room temperature, then washed with PBS for three times. The membrane was incubated with a 1:500 dilution second antibody (peroxidase-conjugate goat-anti-rabbit, Sigma) for one to two hours and washed with PBS as above. Finally, the membrane was colorized with 15 ml of DAB solution (3,3'-Diaminobenzidine Tetrahydrochloride; Fast Dab: Sigma) with gentle agitation until purple dots (positive clones) were visualized. The DAB reaction was stopped by washing the membrane with PBS. The membrane was dried in air for preservation. Plaques corresponding to positive dots in the membrane were marked for further evaluation including purification and isolation. Several single immunologically positive plaques from each of the two cDNA libraries (F5+6 (WB) and F3....6 (FB)) containing small cDNA fragments were used for excision following the single-clone excision protocol described in the ZAP express cDNA synthesis kit (Stratagene). Individual positive plaques obtained from initial screening were further purified and isolated in low concentration of pfu from NZY agar plates and stored in a tube containing 500 microlitres of phage stock buffer (SM buffer) (0.1M NaCl; 0.017 M MgSO4.7H2O, 0.05M Tris-HCl, pH 7.5; 1% (W/V) gelatin, 20 microlitres of chloroform). XL1-Blue MRF' and XLOLR cells were grown separately overnight in NZY broth (5 g of NaCl; 2g of MgSO4.7H2O; 5 g of yeast extract; 10 g of NZ amine with deionized H2O added to a final volume of 1 liter; and pH to 7.5 with NaOH) at 30 degrees Centigrade. Then cells were pelleted and resuspend in 10 mM MgSO4 at a concentration of 1.0 determined spectrophotometry at OD600. First, 200 microlitres of XL1-Blue MRF' cells were mixed with 250 microlitres of the phage stock and 1 microlitre of ExAssist helper phage and the mixture was incubated in a Falcon polypropylene tube at 37 degrees Centigrade for 15 minutes, then 3 ml of NZY broth was added and the solution was incubated for 2.5 -3 hours at 37 degrees Centigrade with shaking. Next, the solution was heated at 65-70 degrees Centigrade for 20 minutes and spun down at 1000 x g for 15 minutes. The supernatant containing the excised pBK-CMV ss DNA phagemid packaged as filamentous phage particles was saved. To get colonies from the phagemid, 200 microlitres of freshly grown XLOLR cells were mixed with 10 microlitres of the excised phagemids. After incubation at 37 degrees Centigrade for 15 minutes, 300 microlitres of NZY broth was added and incubated at 37 degrees Centigrade for another 45 minutes. 200 microlitres of the cell mixture was plated on each LB (loria broth))-kanamycin agar plate and incubated overnight at 37 degrees Centigrade. Next day many colonies would appear on the plates which contain the pBK-CMV double-stranded phagemid vector with the cloned cDNA insert. cDNA was isolated from phagemid using the 'plasmid boiling miniprep protocol' from Stratagene. In general, the **method** for DNA digestion was as follows. A certain amount (2 micrograms) of plasmid DNA was added to a 1.5 ml microcentrifuge tube containing 3 microlitres of universal buffer (Stratagene) was added and then appropriate amount (following recommendation by Stratagene) of restriction enzymes of Xhol and EcoRl were added. The final volume was brought to 20 microllitres with dH2O and incubated at 37 degrees Centigrade for 1 hour. The digested DNA solution was subjected to electrophoresis in 1.0 % agarose gel or stored at -20 degrees Centigrade. Seven out of 30 recombinant plasmids detected by antiserium against Tm 12.86, each containing about 500 base pairs (bps) following digestion by XLo I andEco RI were selected for nucleotide sequencing. These clones were initially sequenced by the dideoxy chain termination **method** using the Sequenase sequencing kit (version 2.0) from U.S. Biochemical Corp. and a 35S-dATP from Du pont

NEN. Both T7 and T3 primers, complementary to the sequence of the vector were used. The purified plasmid DNA was denatured with 0.2 M NaOH containing 0.2 mM EDTA, then neutralized with 0.6 M sodium acetate, pH 5.2 and precipitated with ethanol prior to sequencing. Sequence reaction followed the instruction provided by USB and sequence reaction products (about 3 microlitres) were loaded on 6 % polyacrylamide gel for electrophoresis at a constant power (1500V). After the blue dye reached the bottom of the plate, the gel was placed onto a piece of filter paper and dried under heat (80 degrees Centigrade) and vacuumed on a slab gel drying apparatus. The dried gel was exposed to Fuji X-ray film overnight or longer depending on the count of the radio-**activity** from the monitor. The film was developed according to the instructions provided. After DNA sequence was read, DNA and predicted protein sequences were analyzed with FASTA and Genetics Computer Group version 7.1 programs. Subsequent sequencing was obtained via an automated DNA sequencer.(231 pages)

ACCESSION NUMBER: 2002-07231 BIOTECHDS
TITLE: New cDNA polynucleotide encoding a thermal hysteresis protein which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing antifreeze protection to improve the quality of food;
phagemid vector-mediated recombinat protein gene transfer and expression in bacterium cell, transgenic plant, transgenic fish and transgenic animal for cold climatization enhancement
AUTHOR: HORWATH K L; MYERS K L; EASTON C M
PATENT ASSIGNEE: UNIV NEW YORK STATE RES FOUND; HORWATH K L; MYERS K L; EASTON C M
PATENT INFO: WO 2001094378 13 Dec 2001
APPLICATION INFO: WO 2000-US18532 8 Jun 2000
PRIORITY INFO: US 2000-210446 8 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-090137 [12]

L4 ANSWER 6 OF 17 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI A facile **method** for determining ice **recrystallization inhibition** by antifreeze proteins.
AB Ice recrystallization, the growth of large ice crystals at the expense of small ones, stresses freeze tolerant organisms and causes spoilage of frozen foods. This process is inhibited by antifreeze proteins (AFPs). Here, we present a simple **method** for determining the ice **recrystallization inhibition** (RI) **activity** of an AFP under physiological conditions using 10μl glass capillaries. Serial dilutions were prepared to determine the concentration below which RI **activity** was no longer detected, termed the RI endpoint. For type III AFP this was 200nM. The capillary **method** allows samples to be aligned and viewed simultaneously, which facilitates RI endpoint determination. Once prepared, the samples can be used reproducibly in subsequent RI assays and can be archived in a freezer for future reference. This **method** was used to detect the elution of type III AFP from a Sephadex G-75 size-exclusion column. RI **activity** was found at the expected V(e) for a 7kDa protein and also unexpectedly in the void volume. .COPYRGT. 2003 Elsevier Inc. All rights reserved.
ACCESSION NUMBER: 2003461856 EMBASE
TITLE: A facile **method** for determining ice **recrystallization inhibition** by antifreeze proteins.
AUTHOR: Tomczak M.M.; Marshall C.B.; Gilbert J.A.; Davies P.L.
CORPORATE SOURCE: P.L. Davies, Department of Biochemistry, Protein Eng. Netwk. Centres E., Queen's University, Kingston, Ont. K7L 3N6, Canada. daviesp@post.queensu.ca
SOURCE: Biochemical and Biophysical Research Communications, (28

Nov 2003) 311/4 (1041-1046).
Refs: 21
ISSN: 0006-291X CODEN: BBRCA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

L4 ANSWER 7 OF 17 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Preparation of **antifreeze protein** with reduced glycosylation using a mannosyltransferase deficient Saccharomyces cerevisiae strain
AB A **method** is provided for increasing the specific **activity** of a type III **antifreeze protein** when said protein is prepared by expression in a heterologous fungal species of a gene encoding the protein sequence, by means of reducing the extent of glycosylation of the protein.
ACCESSION NUMBER: 2004:551014 HCAPLUS
DOCUMENT NUMBER: 141:87921
TITLE: Preparation of **antifreeze protein** with reduced glycosylation using a mannosyltransferase deficient Saccharomyces cerevisiae strain
INVENTOR(S): Chapman, John William; Van der Laar, Teun; Lindner, Nigel Malcolm; Visser, Christiaan
PATENT ASSIGNEE(S): Unilever PLC, UK; Unilever NV; Hindustan Lever Limited
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004057007	A1	20040708	WO 2003-EP12219	20031103
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: EP 2002-258921 A 20021220

L4 ANSWER 8 OF 17 HCAPLUS COPYRIGHT 2005 ACS on STN
TI A facile **method** for determining ice **recrystallization inhibition** by antifreeze proteins
AB The authors present a simple **method** for determining the ice recrystn. inhibition (RI) **activity** of an **antifreeze protein** (AFP) under physiol. conditions using 10 μl glass capillaries. Serial dilns. were prepared to determine the concentration below which RI **activity** was no longer detected, termed the RI endpoint. For type III AFP this was 200 nM. The capillary **method** allows samples to be aligned and viewed simultaneously, which facilitates RI endpoint determination Once prepared, the samples can be used reproducibly in subsequent RI assays and can be archived in a freezer for future reference This **method** was used to detect the elution of type III AFP from a Sephadex G-75 size-exclusion column. RI **activity** was found at the expected Ve

for a 7 kDa protein and also unexpectedly in the void volume

ACCESSION NUMBER: 2003:883142 HCAPLUS
DOCUMENT NUMBER: 140:144944
TITLE: A facile **method** for determining ice **recrystallization inhibition** by antifreeze proteins
AUTHOR(S): Tomczak, Melanie M.; Marshall, Christopher B.; Gilbert, Jack A.; Davies, Peter L.
CORPORATE SOURCE: Department of Biochemistry and the Protein Engineering Network of Centres of Excellence, Queen's University, Kingston, ON, K7L 3N6, Can.
SOURCE: Biochemical and Biophysical Research Communications (2003), 311(4), 1041-1046
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 17 USPATFULL on STN
TI Antifreeze proteins from basidiomycetes
AB The present invention provides antifreeze proteins produced by a basidiomycete. The **antifreeze protein** has a high antifreeze **activity** such as a thermal hysteresis **activity** or an ice-**recrystallization inhibition activity**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:257833 USPATFULL
TITLE: Antifreeze proteins from basidiomycetes
INVENTOR(S): Hoshino, Tamotsu, Hokkaido, JAPAN
Kiriaki, Michiko, Hokkaido, JAPAN
Tsuda, Sakae, Hokkaido, JAPAN
Ohgiya, Satoru, Hokkaido, JAPAN
Kondo, Hidemasa, Hokkaido, JAPAN
Yokota, Yuji, Hokkaido, JAPAN
Yumoto, Isao, Hokkaido, JAPAN
PATENT ASSIGNEE(S): NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003180884	A1	20030925
APPLICATION INFO.:	US 2003-386529	A1	20030313 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	JP 2002-72612	20020315
	JP 2003-57888	20030305

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SUGHRUE MION, PLLC, 2100 PENNSYLVANIA AVENUE, N.W., WASHINGTON, DC, 20037
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 1247
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 10 OF 17 USPATFULL on STN
TI COLD TOLERANCES IN PLANTS
AB A plurality of polypeptides derived from intercellular spaces of plant cells having frost tolerance. Some of the polypeptides are ice

nucleators for developing ice crystals in extracellular spaces of plant tissue, some of the polypeptides are antifreeze components which control ice crystal growth in extracellular spaces and some of the polypeptides are enzymes which adapt plant cell walls to function differently during formation of ice crystals in plant intercellular spaces.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:30424 USPATFULL		
TITLE:	COLD TOLERANCES IN PLANTS		
INVENTOR(S):	GRIFFITH, MARILYN, WATERLOO, ONTARIO, CANADA		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003022371	A1	20030130
APPLICATION INFO.:	US 1999-362179	A1	19990727 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1995-485647, filed on 7 Jun 1995, PATENTED Division of Ser. No. US 1995-419061, filed on 10 Apr 1995, PATENTED Continuation of Ser. No. US 1993-60425, filed on 11 May 1993, ABANDONED Continuation-in-part of Ser. No. WO 1992-CA255, filed on 12 Jun 1992, UNKNOWN		
	NUMBER	DATE	
PRIORITY INFORMATION:	GB 1991-12774	19910613	
	GB 1991-26485	19911213	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	SAMUEL G LAYTON JR, BELL SELTZER PARK & GIBSON, POST OFFICE DRAWER 34009, CHARLOTTE, NC, 28234		
NUMBER OF CLAIMS:	23		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	10 Drawing Page(s)		
LINE COUNT:	1580		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 11 OF 17 USPATFULL on STN

TI Nucleic acid sequences encoding type III tenebrio antifreeze proteins and **method** for assaying **activity**

AB Thermal hysteresis proteins and their nucleotide sequences derived from the Tenebrionoidea Superfamily which lower the freezing point of a solution without effecting the melting point. Related methods for preparing said proteins and for providing antifreeze or **recrystallization inhibition** properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:307900 USPATFULL		
TITLE:	Nucleic acid sequences encoding type III tenebrio antifreeze proteins and **method** for assaying **activity**		
INVENTOR(S):	Horwath, Kathleen L., Endwell, NY, UNITED STATES Easton, Christopher M., Ithaca, NY, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002173024	A1	20021121
APPLICATION INFO.:	US 2001-876796	A1	20010607 (9)
	NUMBER	DATE	
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		

LEGAL REPRESENTATIVE: Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St., Binghamton, NY, 13901
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 131 Drawing Page(s)
LINE COUNT: 10082
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 12 OF 17 USPATFULL on STN
TI Nucleic acid sequences encoding type III tenebrio antifreeze proteins and **method** for assaying **activity**
AB A **recrystallization inhibition method** for determining the presence, relative concentration, and/or **activity** of thermal hysteresis proteins comprising: providing a proteinaceous composition in a solvent to form a test solution; flash freezing said solution; raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within said solution; maintaining said frozen solution at the annealing temperature for a length of time sufficient to allow for recrystallization; monitoring the ice crystal grain size changes over time; and determining the presence of functional thermal hysteresis proteins in said solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:307828 USPATFULL
TITLE: Nucleic acid sequences encoding type III tenebrio antifreeze proteins and **method** for assaying **activity**
INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES
Meyers, Kevin L., Trumansburg, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002172951	A1	20021121
APPLICATION INFO.:	US 2001-876348	A1	20010607 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St., Binghamton, NY, 13901
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 131 Drawing Page(s)
LINE COUNT: 10121
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 13 OF 17 USPATFULL on STN
TI Cold tolerances in plants
AB A plurality of polypeptides derived from intercellular spaces of plant cells having frost tolerance. Some of the polypeptides are ice nucleators for developing ice crystals in extracellular spaces of plant tissue, some of the polypeptides are antifreeze components which control ice crystal growth in extracellular spaces and some of the polypeptides are enzymes which adapt plant cell walls to function differently during formation of ice crystals in plant intercellular spaces.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:132568 USPATFULL
TITLE: Cold tolerances in plants

INVENTOR(S): Griffith, Marilyn, Waterloo, Canada
PATENT ASSIGNEE(S): University of Waterloo, Ontario, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5972679		19991026
APPLICATION INFO.:	US 1995-485647		19950607 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-419061, filed on 10 Apr 1995, now patented, Pat. No. US 5852172 which is a continuation of Ser. No. US 1993-60425, filed on 11 May 1993, now abandoned which is a continuation-in-part of Ser. No. WO 1992-CA255, filed on 12 Jun 1992

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1991-12774	19910613
	GB 1991-26485	19911213

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Weber, Jon P.
LEGAL REPRESENTATIVE: Alston & Bird LLP
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 27 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 1673
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 14 OF 17 USPATFULL on STN
TI Cold tolerances in plants
AB A plurality of polypeptides derived from intercellular spaces of plant cells having frost tolerance. Some of the polypeptides are ice nucleators for developing ice crystals in extracellular spaces of plant tissue, some of the polypeptides are antifreeze components which control ice crystal growth in extracellular spaces and some of the polypeptides are enzymes which adapt plant cell walls to function differently during formation of ice crystals in plant intercellular spaces.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:160102 USPATFULL
TITLE: Cold tolerances in plants
INVENTOR(S): Griffith, Marilyn, Waterloo, Canada
PATENT ASSIGNEE(S): University of Waterloo, Ontario, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5852172		19981222
APPLICATION INFO.:	US 1995-419061		19950410 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-60425, filed on 11 May 1993, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1991-12774	19910613
	GB 1991-26485	19911213

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Weber, Jon P.
LEGAL REPRESENTATIVE: Bell Seltzer Intellectual Property Law Group of Alston & Bird LLP
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 30 Drawing Figure(s); 12 Drawing Page(s)

LINE COUNT: 1529
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 15 OF 17 USPATFULL on STN
TI Transgenic plants having a nucleic acid sequence encoding a dendroides **antifreeze protein**
AB The present invention is directed to transgenic plants having nucleic acid sequences encoding Dendroides canadensis thermal hysteresis proteins. The THPs of Dendroides have significantly greater thermal hysteresis **activity** than any other known anti-freeze protein. The thermal hysteresis **activity** of the purified THPs can be further enhanced by combining the THPs with various "activating" compounds.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 97:45207 USPATFULL
TITLE: Transgenic plants having a nucleic acid sequence encoding a dendroides **antifreeze protein**
INVENTOR(S): Duman, John G., South Bend, IN, United States
PATENT ASSIGNEE(S): University of Notre Dame du Lac, Notre Dame, IN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5633451		19970527
APPLICATION INFO.:	US 1995-569594		19951208 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-485359, filed on 7 Jun 1995
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Fox, David T.
ASSISTANT EXAMINER: Haas, Thomas
LEGAL REPRESENTATIVE: Barnes & Thornburg
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 966
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 16 OF 17 USPATFULL on STN
TI Nucleic acid sequences encoding dendroides antifreeze proteins
AB The present invention is directed to nucleic acid sequences encoding Dendroides canadensis thermal hysteresis proteins. The THPs of Dendroides have significantly greater thermal hysteresis **activity** than any other known anti-freeze protein. The thermal hysteresis **activity** of the purified THPs can be further enhanced by combining the THPs with various "activating" compounds.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 97:38394 USPATFULL
TITLE: Nucleic acid sequences encoding dendroides antifreeze proteins
INVENTOR(S): Duman, John G., South Bend, IN, United States
PATENT ASSIGNEE(S): University of Notre Dame du Lac, Notre Dame, IN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5627051		19970506
APPLICATION INFO.:	US 1995-485359		19950607 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jacobson, Dian C.

ASSISTANT EXAMINER: Lau, Kawai
LEGAL REPRESENTATIVE: Barnes & Thornburg
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 959
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 17 OF 17 USPATFULL on STN
TI Ice crystal growth suppression polypeptides and **method** of making
AB Novel methods of improving freezing tolerance of organic materials through the use of antifreeze polypeptides is provided. These polypeptides increase the storage life of foodstuffs and biologics, as well as protect plant products, such as during growth. The antifreeze polypeptides, or their fusion proteins, may be produced chemically or by recombinant DNA techniques, and then purified for a variety of uses.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 92:44933 USPATFULL
TITLE: Ice crystal growth suppression polypeptides and **method** of making
INVENTOR(S): Warren, Gareth J., San Francisco, CA, United States
Mueller, Gunhild M., San Francisco, CA, United States
McKown, Robert L., Albany, CA, United States
PATENT ASSIGNEE(S): DNA Plant Technology Corporation, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5118792		19920602	
APPLICATION INFO.:	US 1989-350481		19890510	(7)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Robinson, Douglas W.
ASSISTANT EXAMINER: Weber, Jon P.
LEGAL REPRESENTATIVE: Townsend and Townsend
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 30 Drawing Figure(s); 29 Drawing Page(s)
LINE COUNT: 1850
CAS INDEXING IS AVAILABLE FOR THIS PATENT.